# Gonococcal Isolate Surveillance Project (GISP)

# Protocol

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U.S. DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

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## Background

Antimicrobial resistance in *Neisseria gonorrhoeae* has been due either to multiple chromosomal mutations or to R-factor plasmids. The development of plasmid-mediated resistance to tetracycline, i.e., TetM, prompted CDC to recommend, in 1985, that tetracycline not be used for the treatment of gonococcal infections. In addition, the increasing prevalence of strains with plasmid-mediated resistance to penicillin (PPNG) prompted the virtual abandonment of penicillins as single-dose therapies for gonorrhea in 1987. Isolates with chromosomal resistance to alternative drugs such as spectinomycin have also been reported. Most recently, resistance to fluoroquinolones has emerged.

The Gonococcal Isolate Surveillance Project (GISP) was established in 1986 to monitor trends in antimicrobial susceptibilities of strains of *N. gonorrhoeae* in the United States and to establish a rational basis for the selection of gonococcal therapies. Approximately 27 cities participate in GISP. Data from this project have been reported and used to revise the CDC's STD Treatment recommendations in 1989, 1993, and 1998. This protocol supersedes all previous descriptions of the project.

## Objectives

1. To monitor trends in antimicrobial susceptibilities in *N. gonorrhoeae.*

2. To phenotypically characterize antimicrobial-resistant isolates to describe the diversity of antimicrobial resistance in *N. gonorrhoeae*.

## Methods

The Gonococcal Isolate Surveillance Project is a collaborative project between the CDC (Epidemiology and Surveillance Branch [ESB] and Statistics and Data Management Branch [SDMB], Division of STD Prevention [DSTDP], National Center for HIV, STD, and TB Prevention [NCHSTP]; and the Bacterial STD Branch [BSTDB], Division of AIDS, STD, and TB Laboratory Research [DASTLR], National Center for Infectious Diseases [NCID]), five regional laboratories, and selected STD clinics. The responsibilities of each group of participants are detailed in this protocol.

## Sentinel Sites

The GISP system is composed of sentinel STD clinics located in geographically diverse cities in the United States, and five regional laboratories. GISP analyses are based on demographic and clinical data from the first 20 male patients attending the sentinel clinics each month with a urethral gonococcal infection supported by a positive culture for *N. gonorrhoeae*, and on antimicrobial susceptibility testing of urethral isolates obtained from these patients. Most isolates will be pre-treatment isolates; however, post-treatment isolates may be included in the sample. A total of 25 isolates and corresponding patient demographic/clinical data will be collected monthly at each site (when possible) in order to ensure that data from 20 patients will be available.

At each Sentinel Site, an individual will be assigned to be responsible for data and appropriate specimen/isolate collection, and to ensure that the isolates are sent to the Regional Laboratories and the epidemiologic data are sent to the Regional Laboratories and to CDC.

To participate in GISP, those sentinel STD clinics that regularly use non-culture methods for gonococcal testing can routinely use gonorrhea culture in lieu of or in addition to non-culture testing on all or a subset of patients. Culture provides useful data (i.e., data on antimicrobial susceptibility) that can benefit patients directly and is important for local disease control efforts. *(Revised 2/2000)*

**Isolate Collection, Handling, and Shipping**

1. Urethral isolates of *N. gonorrhoeae* (based on a presumptive* or confirmed identification) will be collected from the first 25 men with urethral gonococcal infection each month. Usually, the isolates will be collected starting on the Monday of the first full week each month. For some sites, more than one full business week may be required to collect 25 isolates.

   *A presumptive identification of *N. gonorrhoeae* will be based on the following criteria: (i) growth of typical appearing colonies on a selective medium such as Thayer-Martin at 35 C to 36.5 C in 5% $CO_2$, (ii) a positive oxidase test, and (iii) the observation of gram-negative, oxidase-positive diplococci in stained smears.

2. Gonococcal isolates will be subcultured from the selective primary medium to a noninhibitory medium, e.g., chocolate agar with 1% IsoVitaleX to obtain a pure culture of the isolate. If the subcultured isolate is not pure, serial subcultures of individual colonies must be performed until a pure culture is obtained. After 18 to 20 h. incubation, growth from the pure culture is suspended heavily in trypticase soy broth containing 20% (v/v) glycerol; duplicate frozen cultures of each isolate are prepared.

   Isolates will be assigned sequential identifiers for each month. Each identifier will be composed of a three-letter designation for the Sentinel Site, followed by a six-digit number indicating the year and month (yyyymm), and a two digit number in the sequence from 01 through 25. For example, the 20th isolate selected in January 1990 in Atlanta will be given the number ATL-199001-20.

3. Isolates will be frozen to -70 C if possible. If a -70 C freezer is not available, isolates may be frozen to -20 C (freezer/dry ice chest) until shipped to the Regional Laboratory; isolates must be placed in the coldest sections of the -20 C freezer (not in the door or at the front of a shelf). A frost-free freezer should not be used.

4. Isolates should be shipped each month to the Regional Laboratory** on Monday of the week immediately following completion of collection of the isolates but no later than the first Monday of the month following the month of isolate collection. Duplicate isolates must be kept until the Regional Laboratory confirms that viable isolates have been received.

** Please telephone the Regional Laboratory **prior** to shipping the strains to confirm when the strains will be shipped and to ensure that someone in the Regional Laboratory will be available to receive the strains. Ideally, isolates should be shipped no later than Wednesday in any week in which they are shipped to ensure that they are received at the Regional Laboratory before close of business on Friday. Isolates must be packed in two leak-proof containers, one inside the other. The package containing the isolates should be packed in insulated styrofoam containers (to be provided by the Regional Laboratory) with dry ice (at least 10 lb); dry ice should be packed on each side of the package of isolates. Ship the container by overnight express, charging the shipping costs to the account number provided by the Regional Laboratory. (The container will be returned to the Sentinel Site by regular package delivery for future shipments). Sites should ship GISP isolates ***each month***; isolates should not accumulate for several months and then be shipped together because this prevents the Regional Laboratories from completing the susceptibility testing on schedule.

**Reporting of Demographic and Clinical Data by Sentinel Sites**

***Patient Data***

Demographic and clinical data should be submitted for each patient from whom an isolate is selected. A unique number will be assigned to each patient (see Isolate Collection, item 2 above). Data may be obtained through review of medical records by the clinic staff. Data will be entered on the triplicate Form 1 (CDC 73.60 A) using the GISP Coding Guide provided by DSTDP, NCHSTP. The original copy of Form 1 should be mailed to the GISP Data Manager, Statistics and Data Management Branch SDMB, DSTDP, Mailstop E-63, Centers for Disease Control and Prevention, Atlanta, GA 30333; one of the carbon copies should be mailed to the Regional Laboratory, and the other carbon copy should be maintained in the clinic files. As an alternative, data may be submitted in electronic form (via diskette) using data entry files provided by the GISP data manager. Clinics submitting data electronically may discontinue hard copy reports once equivalency is established by the GISP data manager (see Appendix 6 for Description of Data Elements).

Demographic and clinical data reports should be received at CDC *no more than four weeks* after the end of the month in which the corresponding isolates were collected.

The demographic and clinical data to be collected are as follows:

Clinic code
Sequential patient number (01-25)
Sex
Race (census categories)
Ethnicity
Date of clinic visit
Date of birth
Age
Sexual orientation
Symptoms
Reason for clinic visit
Primary treatment (for gonorrhea)
Secondary treatment (co-treatment for presumed chlamydia)
Previous history of gonorrhea
Number of previous confirmed episodes of gonorrhea in past year
Zipcode

***Clinic Data***

In addition, each Sentinel Site will submit with their monthly report the total number of episodes of gonorrhea that were diagnosed at the clinic in the current or previous month, as well as the subtotals of number of episodes of gonorrhea diagnosed in men and in women.

**Sentinel Site Annual Reporting**

Sentinel Sites should report annually, in the STD grant application, on the following items:

1. Enrollment strategy: Is GISP capturing the first 25 men with urethral gonorrhea seen at the clinic each month? (Are all men cultured, at least until 25 positive cultures obtained? Is sample defined by first 25 positive cultures, and not by Gram stains or other ways?);

2. Percentage of isolates which are dead or contaminated (list separately) on receipt at Regional Laboratory (will be reported to Sentinel Sites by Regional Laboratories);

3. Percentage of clinical and demographic data that is incomplete or missing (will be reported to site by CDC);

4. Timeliness of isolate and data submission (isolates should be shipped to lab within 1 week after end of collection and data should be sent within 4 weeks after end of month in which corresponding isolates are collected);

5. Laboratory procedures for isolate processing prior to shipping;

6. Storage of duplicate isolates until Regional Laboratory confirms that viable isolates have been received.

## Regional Laboratories

The Regional Laboratories in Atlanta, Birmingham, Cleveland, Denver, and Seattle will determine ß-lactamase production and antimicrobial susceptibilities of isolates within 30 calendar days from the date of receipt of the isolates from the Sentinel Sites.

**Receipt of Isolates:** The isolates will be cataloged and frozen at -70 C until tested. Any problems with the isolates such as improper shipping or contamination should be reported as soon as possible to the Sentinel Site and to CDC. The insulated containers will be returned to the Sentinel Sites for future shipments by regular mail.

**Confirmatory Testing:** The Regional Laboratories are not required to perform confirmatory tests on all isolates although it is recommended that confirmatory tests should be performed on any isolate that exhibits atypical colonial morphologic characteristics or aberrant susceptibility patterns. Because we are testing only urethral isolates, we do not anticipate a significant problem with the inclusion of nongonococcal isolates in the sample. However, urethral *N. meningitidis* isolates may be isolated from homosexual men with greater frequency than from heterosexual men. Thus, nongonococcal strains may be isolated more frequently in patient populations with a high proportion of homosexual men. Because gonococcal serologic reagents may cross-react with nongonococcal *Neisseria* and related species, we recommend that strains be identified with tests that detect acid production from carbohydrates and/or enzyme substrate tests.

**ß-Lactamase Tests:** All isolates will be tested for ß-lactamase by the nitrocefin or an equivalent test. The PADAC test may be substituted for the nitrocefin test (see Appendix 2).

**Antimicrobial Susceptibility Testing:** Antimicrobial susceptibilities (minimal inhibitory concentrations, MICs) to penicillin G, tetracycline, spectinomycin, cefixime, ceftriaxone, ciprofloxacin, and azithromycin will be determined by the agar-dilution procedure on GC II agar base (Becton Dickinson, Cockeysville, MD) inoculated with $10^4$ colony forming units (CFU) (see Appendix 3). Erythromycin is an optional agent for antimicrobial susceptibility testing. Regional Laboratories should include a set of control strains (F-18, F-28, F-45, P681E, CDC 10328, and CDC 10329) with each run and report control strain MIC data on Form 3 (CDC 73.60C) each month. The original copy of Form 3 should be mailed to GISP Data Manager, SDMB, DSTDP, Mailstop E-63, NCHSTP, Centers for Disease Control and Prevention, Atlanta, GA 30333 and the copy should be maintained in the laboratory files. It is expected that susceptibility testing will be completed within one month of receipt of isolates from a Sentinel Site, and reported to CDC on a monthly basis within one week of completion.

If isolates having MICs higher than those listed below are identified, it is the responsibility of the Regional Laboratory to retest these isolates to confirm the high MICs. Regional Laboratory personnel may retest these isolates with the next batch of isolates, provided this is no longer than one month after the initial test.

MICs requiring repeat testing:

Ceftriaxone: MIC > 0.25 Fg/ml
Cefixime: MIC > 0.25 Fg/ml
For ceftriaxone and cefixime, isolates should be tested for growth on medium containing the antibiotics at concentrations ranging from two dilutions below the initial MIC to two dilutions above the initial MIC, to reach an endpoint MIC.

Ciprofloxacin: MIC > 0.06 Fg/ml
For ciprofloxacin, isolates should be tested for growth on medium containing two-fold dilutions of ciprofloxacin up to, and including, 32.0 Fg/ml of ciprofloxacin, to reach an endpoint MIC.

Azithromycin: MIC >1.0 Fg/ml

For azithromycin, isolates should be tested for growth on medium containing azithromycin at concentrations ranging from two dilutions below the initial MIC to two dilutions above the initial MIC, to reach an endpoint MIC.

Spectinomycin: MIC >128 Fg/ml

For spectinomycin, isolates should be retested for growth on 128.0 Fg/ml of spectinomycin.

**Isolates with High MICs:** Isolates with the following MICs should be confirmed by retesting:

Cefixime or ceftriaxone: MIC >0.5 Fg/ml
Ciprofloxacin: MIC $1.0 Fg/ml *(revised, 7/1999)*
Azithromycin: MIC >1.0 Fg/ml *(revised, 7/1999)*
Spectinomycin: MIC >128 Fg/ml

ESB, DSTDP, NCHSTP, CDC and the Sentinel Site that submitted the isolate(s) should be notified **immediately** by telephone or by e-mail of any isolate(s) confirmed to have a high MIC. Such isolates also should be shipped rapidly to CDC; laboratory personnel should not wait until the request lists arrive from CDC to ship these isolates. A sheet marked "Attn: High MICs" that contains the original and the retest MIC values for each isolate should be included with the isolate shipment; a copy of this same sheet should **also** be sent separately to the GISP Data Manager.

**Isolate Preservation:** All isolates will be suspended, in duplicate, in trypticase soy broth containing 20% (v/v) glycerol and frozen at -70 C in duplicate at the Regional Laboratories. When isolates are selected for further characterization, duplicate copies of each isolate must be maintained at the Regional Laboratory until notified by BSTDB, DASTLR, NCID, CDC that the isolates have been received. This will generally require maintenance of isolates from the current year and the previous year.

**Data reporting:** Upon completion of laboratory testing, the antimicrobial susceptibilities for isolates submitted from each Sentinel Site will be entered on triplicate Form 2 (CDC 73.60 B). Instructions for coding are found in the GISP Coding Guide. The original copy of Form 2 should be mailed to GISP Data Manager, SDMB, DSTDP, Mailstop E-63, NCHSTP, Centers for Disease Control and Prevention, Atlanta, GA 30333; one of the carbon copies should be mailed to the Sentinel Site, and the other carbon copy should be maintained in the laboratory files. As an alternative, data may be submitted in electronic form (via diskette) using data entry files provided by the GISP data manager. Laboratories submitting data electronically may discontinue hard copy reports once equivalency is established by the GISP data manager (see Appendix 6 for Description of Data Elements).

**Proficiency Testing:** A set of 60 coded isolates will be provided to the Regional Laboratories by BSTDB, DASTLR, NCID, CDC for antimicrobial susceptibility testing annually. These isolates will include strains selected to represent susceptible and resistant isolates of *N. gonorrhoeae* and may include duplicate samples of some strains. Isolates should be tested and results reported to CDC within 60 days of receipt. CDC will report back to the laboratories with a preliminary discussion of results within 30 days of receiving results from all sites. If the proficiency testing results suggest problems in MIC testing, the laboratory should identify and address these problems, and report to CDC on corrections made. This report should be made within 30 days of notification by CDC that corrective actions are necessary. A second set of proficiency isolates will then be provided for testing, with another 60 days for completion. If proficiency problems cannot be solved, testing may have to be shifted to an alternate laboratory.

**Training and Consultation for Sentinel Sites:** In addition to performing laboratory studies on isolates, the Regional Laboratories will be asked to perform appropriate training of or consultation with laboratory personnel from Sentinel Sites. To assist Sentinel Sites in addressing any problems with isolate storage or shipment, Regional Laboratories will be asked to report annually to each of their Sites the number and percentage of isolates that were nonviable or grossly contaminated with other organisms (separately) on receipt. If a problem with nonviability or contamination is recognized by the Regional Laboratory, this should be brought to the attention of the Sentinel Site

immediately, without waiting for the annual reporting.

**Regional Laboratory Annual Reporting**
In addition to annual reporting to the Sentinel Sites as described above, Regional Laboratories should report annually, in the STD grant application, on the following items:

1. Timeliness of isolate testing (should be completed within 30 days of receipt) and data submission (should be on monthly basis).

2. Storage of duplicate isolates when isolates are requested by CDC, in case any follow-up testing at the regional lab is needed.

3. Use of control strains and reporting of control strain MIC data.

4. Proficiency testing results.

## Centers for Disease Control and Prevention

The administrative and functional duties relating to GISP will be performed in ESB, SDMB, and Program, Development and Support Branch [PDSB], DSTDP, NCHSTP, and BSTDB, DASTLR, NCID. The recruitment of future Sentinel Sites as well as the initiation of data analyses will be a dual function of these divisions.

**DSTDP, NCHSTP:**

1. Site visits, as needed, to Sentinel Sites and Regional Laboratories.

2. Implementation of the demographic/clinical protocols, including reporting to Sentinel Sites the percentage of the individual Site's data that are incomplete or missing.

3. Management and analysis of demographic, clinical, and antimicrobial susceptibility data.

4. Preparation and distribution of regional and site-specific data in electronic format on a per request basis.

5. In consultation with BSTDB, DASTLR, NCID, preparation and distribution of annual reports summarizing study findings.

**DASTLR, NCID:**

1. Coordination with Regional Laboratory operations.

2. Training of Regional Laboratory personnel when necessary.

3. Selection and distribution of reference strains and GC II agar base medium for antimicrobial susceptibility testing.

4. Distribution of quality control isolates.

5. Molecular epidemiologic characterization of selected isolates (PPNG, TRNG, PPNG/TRNG, spectinomycin-resistant isolates, isolates with decreased susceptibility to ciprofloxacin [MIC $0.06 Fg/ml] or relatively high MICs to ceftriaxone [MIC $0.06 Fg/ml], others as deemed appropriate).

6. Identification of novel antimicrobial susceptibility patterns among isolates that require further investigation.

The duties listed above may overlap in many areas. Frequent communications of Principal Investigators, Co-Investigators, and Associates are conducted to monitor the day-to-day activities of the project. Policy meetings of Principal Investigators and Co-Investigators are conducted as required.

## Quality Assurance

It is expected that clinic sites will perform the tasks described in this protocol in a timely and efficient manner within the prescribed deadlines. Problems in adhering to the protocol at the Sentinel Sites should be reported to the Regional Laboratories; problems at the Regional Laboratories should be referred to the appropriate contact at the CDC.

## Local and Regional Use of Data and Isolates

Local and regional use of GISP data are encouraged. To promote quality and standardization, and as a courtesy to those involved in the project, draft manuscripts that use GISP data should be distributed to all GISP investigators for review and comment.

GISP isolates are collected primarily for the purposes stated in the GISP protocol, but some uses of isolates not described in this protocol may be desirable and may enhance the public health usefulness of this project. To ensure adequate communication and address any human subjects issues which may arise with the research use of isolates collected for public health surveillance, uses not described in this protocol should be initiated through the following process: 1) a brief written proposal should be produced, 2) consent and/or collaboration of CDC investigators should be obtained, 3) consent and/or collaboration of the appropriate Regional Laboratory investigator should be obtained, 4) consent and/or collaboration of the Sentinel Sites that provided isolates should be obtained, and 5) Institutional Review Board (IRB) review should be sought as appropriate. Submission to CDC is requested as a first step to ensure that projects do not overlap work already in progress and to determine the need for IRB review.

An exception to this process is isolates which are already collected dually under GISP and another ongoing protocol. In that case, appropriate consents and/or collaborations of the persons collecting and processing the isolates should already have been obtained. Local IRB review should be sought as appropriate.

## Project Expansion - Selection Criteria

The GISP system may be expanded to include additional geographic sites; these sites will be chosen based on geographic location, patient population characteristics, clinic operating procedures, clinic volume, and the ability of the clinic and laboratory personnel to adhere to the technical and time-limit requirements of the protocol.

# Appendix 1

**Form 1**

**Demographic/Clinical Data**

# Appendix 2

### ß-Lactamase Testing

ß-Lactamase may be detected with a chromogenic cephalosporin test. Either a nitrocefin disk test or, alternatively, the PADAC test may be used.

PADAC is available from Calbiochem (Catalog No. 506302). A solution of 25.0 Fg/ml may be prepared in distilled water; the solution may be stored at 4 C for several weeks although it is recommended that the solution be stored in small aliquots (1-2 ml) at -70 C. The test is best performed in a tube or microtiter plate. Suspend organisms heavily in approximately 0.25 Fl contained in a tube or microtiter plate well. A color change from purple to salmon orange occurs if a strain is ß-lactamase positive; the change should occur within 15 secs with an 18 to 24 h culture.

# Appendix 3

## Antimicrobial Susceptibility Testing

**Antimicrobial Agents and Range of dilutions (Fg/ml):**

| | |
|---|---|
| **Standard panel:** | Penicillin G: 0.008 to 32.0<br>Tetracycline: 0.06 to 32.0<br>Spectinomycin: 128.0<br>Ceftriaxone: 0.001 to 1.0<br>Ciprofloxacin: 0.001 to 4.0<br>Cefixime: 0.002 to 2.0<br>Azithromycin: 0.008 to 4.0 |
| **Optional agent:** | Erythromycin: 0.008 to 4.0 |

**Retest criteria:**
If the MICs of strains are not determined at the highest concentration of agent tested, the MIC should be retested for susceptibility to a higher range of two-fold dilutions. An endpoint must be determined. For specific repeat testing criteria, see pages 10-11.

## Preparation of Antibiotic-Containing Media

GC II agar base medium (Becton Dickinson, Cockeysville, Md.) supplemented with 1% IsoVitaleX (or an equivalent supplement) is used.

1. Prepare the required volume of GC base medium in single strength according to the manufacturer's directions.
2. Autoclave the medium at 121 C for 15 min. Cool to 50 C in a waterbath.
3. Reconstitute the dehydrated IsoVitaleX with the provided diluent according to the manufacturer's directions.
4. Add 10 ml of supplement per liter of base medium i.e., 1% (v/v); mix thoroughly. This medium is GCS medium.
5. Dispense the required volume of medium into individual containers for the addition of antimicrobial solutions. Maintain media at 50 C in a waterbath.
6. Prepare the working solutions and dilutions of antimicrobial agents from the stock solutions or standard powder.

**Note:** *It is important that no longer than 1 hour elapse between the time that the stock solution is thawed, the dilutions are prepared and added to the base medium and the plates are poured.*

7. Add the required volumes of the prepared working solutions and dilutions of the antimicrobial agents to the respective bottles of GCS medium, mix thoroughly and dispense into *clearly labeled* plates. Thorough mixing of the antibiotics in the medium can be accomplished by swirling the contents three times in a clockwise and counterclockwise motion followed by inverting the bottle three times, minimizing bubble formation.
8. Allow the plates to cool. *Invert the plates and store them in sealed plastic bags at 4 C to 8 C for no longer than two weeks prior to use.*

**Agar-Dilution Susceptibility Test Procedure**

1. Grow pure cultures of isolates to be tested on chocolate agar at 35 C to 36.5 C for 16 to 18 h in a $CO_2$-enriched (5%) atmosphere. Use pure cultures on chocolate agar; *do not use the first subculture from a frozen culture; subculture these isolates again before testing. Do* ***not*** *test isolates grown on antibiotic-containing media.* Four control organisms provided by the CDC should be included in each run.

2. Use a cotton applicator or a bacteriologic loop to suspend isolated colonies (or cells from less dense areas of growth on the plate) in approximately 2 ml of Mueller-Hinton (MH) broth.

   *(The exact volume of broth required will depend on the method for measuring the turbidity of the suspension. If a spectrophotometer is used, the volume of broth must be sufficient to completely cover the light path and will vary according to the type of spectrophotometer).*

3. Adjust the density of the suspension to contain $10^8$ colony forming units (CFU)/ml by comparison with a 0.5 McFarland $BaSO_4$ turbidity standard.

   *(If a spectrophotometer is used to measure the optical density of the suspensions, set the wavelength at 450 nm. Adjust the turbidity to approximately 0.15. It may be necessary to dilute this suspension further than indicated in step #4. Determine the viable count for the suspension and either adjust the initial optical density to which the suspension is prepared or the dilution to give a final viable count of $10^7$ CFU/ml as indicated in step #4.)*

4. Dilute this suspension 1:10 in MH (or equivalent) broth to give $10^7$ CFU/ml.

5. Dispense an equal volume of each suspension into wells of a replicating device, e.g., Steer's or Cathra replicators. These replicating devices deliver 0.001 - 0.005 ml of the bacterial suspension to the surface of the medium, i.e., $10^4$ CFU.

6. Inoculate each plate of the set of antibiotic containing media plus a plate of chocolate agar or GCS medium (as a control to determine that all isolates grew). You may also wish to inoculated a GCS plate between each set of antibiotic-containing medium to ensure against carry-over of antimicrobial agents from one medium to another; these plates also allow for monitoring for contamination of the inocula during the inoculation process.

**Note.** *The time elapsing between the preparation of the strain suspensions and inoculation of the plates should not exceed 1 h.*

7. Allow the inoculated plates to air dry at room temperature for approximately 15 min. Invert the plates incubate at 35 C to 36.5 C in a $CO_2$-enriched (5%) atmosphere for 24 h.

8. Examine the plates for growth. Use a separate sheet to record the results for each antibiotic tested and record the growth for each isolate on each antibiotic concentration tested. Record the growth as good (+), poor (±), or no growth (-). By using this scheme, the results can be reviewed at a later date for transcription errors and, when isolates grow on the highest concentration tested, the degree of growth will indicate whether the isolate is growing strongly or is partially inhibited.

**Agar Dilution Susceptibility Testing--CDC Reference Strains of *Neisseria gonorrhoeae***

**MICs (Fg/ml)**

| in # | Resistance Phenotype* | Pen Range (Median) | Tet Range (Median) | Spc Range (Median) | Ery Range (Median) | Cro Range (Median) | Cfx Range (Median) | Cip Range (Median) | Azi Rang (Media |
|---|---|---|---|---|---|---|---|---|---|
| 3 | Susceptible | 0.5-1.0 (1.0) | 05-1.0 (.5-1.0) | #128.0 (#128.0) | 1.0-2.0 (2.0) | 0.004-0.015 (0.015) | 0.015-0.06 (0.03-0.06) | #0.002 (#0.002) | 0.25-( |
| 3 | SpcR | 0.015-0.03 ( 0.03) | 0.125-0.25 (0.125-0.25) | >128.0 (>128.0) | 0.125 (0.125) | 0.0005-0.004 (#0.002) | #0.002-0.004 (0.004) | #0.002 (0.002) | #0.( |
| 5 | CMRNG | 8.0-16.0 (8.0-16.0) | 4.0-8.0 (4.0-8.0) | #128.0 (#128.0) | 2.0-4.0 (2.0) | 0.125-0.25 (0.125) | 0.06-0.25 (0.125-0.25) | 0.015-0.03 (0.015) | 0.25-( |
| 1E | PP/TR | 2.0-8.0 (4.0-8.0) | 4.0-16.0 (16.0) | #128.0 (#128.0) | 0.06-0.125 (0.125) | 0.002-0.008 (0.004) | 0.008-0.03 (0.015) | <0.001-0.002 (0.002) | #0.03-0. (0.0 |
| C10328** | PPNG,CipI | $32.0 ($32.0) | 0.5-1.0 | #128.0 (#128.0) | 0.5 | 0.004-0.015 | 0.008 | 0.25-0.5 (0.25) | 0.0 (0.0 |
| C10329** | PPNG, TetR, CipR | $32.0 ($32.0) | 2.0-4.0 (2.0) | #128.0 (#128.0) | 1.0 | 0.008-0.015 (0.015) | 0.015 (0.015) | 1.0 (1.0) | 0.2 |

**previations.** SpcR, spectinomycin-resistant; CMRNG, MICs of $2.0 Fg/ml for both penicillin and tetracycline; PP/TR, ß- lactamase-positive ins which possess the 25.2-megadalton conjugative plasmid containing the TetM determinant; CipI, strain with MIC of 0.125-0.5 Fg/ml of ofloxacin; TetR, strain with MIC of $2.0 Fg/ml of tetracycline; CipR, strain with MIC of $1.0 Fg/ml of ciprofloxacin.

se values are provided as guidelines for agar dilution susceptibility testing; values may differ slightly due to interlaboratory variability.

sistance phenotype for penicillin and tetracycline, except where noted e.g., SpcR, spectinomycin-resistant; CipI, intermediate resistance (MI 5-0.5 Fg/ml); CipR, ciprofloxacin resistant (MIC $1.0 Fg/ml).
esults are preliminary and may be adjusted at a later date. Median MICs not provided unless a pattern is obvious.

P Protocol: May 1999

# Appendix 4

**Form 2**

**Antimicrobial Susceptibility Data Report Form**

# Appendix 5

**Form 3**

**Control Strain Susceptibility Testing**

## Appendix 6

## Description of Data Elements

## GISP Data Elements

Demographic/Clinical Data (Form 1 or CDC 73.60A)

| Variable Name | Type/Length | Description | Values |
|---|---|---|---|
| CLINIC | [Char,3] | Sentinel site code | ALB=Albuquerque, ANC=Anchorage, ATL=Atlanta, BAL=Baltimore, BHM=Birmingham, CHI=Chicago, CIN=Cincinnati, CLE=Cleveland, DEN=Denver, FBG=Fort Bragg, FLW=Fort Lewis, HON=Honolulu, KCY=Kansas City, LBC=Long Beach, MIA=Miami, MIN=Minneapolis, NAS=Nassau County, NOR=New Orleans, ORA=Orange County, PHI=Philadelphia, PHX=Phoenix, POR=Portland, SDG=San Diego, SEA=Seattle, SFO=San Francisco, STL=St. Louis, STO=San Antonio, WPB=West Palm Beach |
| YRMO | [Char,6] | Year/Month of patient's visit | YYYYMM |
| TOTYRMO | [Char,6] | Year/Month of clinic totals for gonorrhea | YYYYMM |
| EPFEM | [Num,3] | monthly female gc episodes | |
| EPMALE | [Num,3] | monthly male gc episodes | |
| TOTEP | [Num,3] | monthly total gc episodes | |
| ID | [Char,2] | patient or isolate number | 01, 02, 03, ... 25 |
| SITE | [Char,1] | clinic identifier number | |
| SEX | [Char,1] | gender | 1=male, 2=female, 9=unknown |
| RACE | [Char,1] | race | 1=white, 2=black, 4=Asian/Pacific Islander, 5=Native Amer/Alaskan, 8=Other, 9=Unk |
| ETHNIC | [Char,1] | Hispanic origin | 1=Hispanic, 2=non-Hispanic, 9=Unknown |
| DATEVIS | [Date,10] | date of clinic visit | MM/DD/YYYY |
| DOB | [Date,10] | date of birth | MM/DD/YYYY |
| AGE | [Num,2] | age in years | |
| SEXOR | [Char,1] | sexual orientation | 1=heterosexual, 2=homosexual, 3=bisexual, 9=unknown |
| SYMP | [Char,1] | symptoms of gonorrhea | 1=discharge and/or dysuria, 2=no discharge or dysuria, 9=symptoms unknown |

| Variable Name | Type/Length | Description | Values |
|---|---|---|---|
| REASON | [Char,1] | reason for clinic visit | 1=volunteer, 2=contact of gonorrhea patient, 3=test of cure, 8=other, 9=unknown |
| TRMT1 | [Char,2] | primary treatment for gc | 00=none, 03=spectinomycin (Trobicin), 04=ceftriaxone (Rocephin) 250 mg., 05=ceftriaxone (Rocephin) 125 mg., 06=ciprofloxacin, 07=cefoxitin (Mefoxin), 12=cefixime (Suprax), 14=cefpodoxime proxetil, 15=ofloxacin (Floxin), 17=ceftizoxime (Cefizox), 18=cefotaxime (Claforan), 19=cefotetan (Cefotan), 20=cefuroxime axetil (Ceftin), 88=other (please indicate in othtrmt1), 99=unknown |
| OTHTRMT1 | [Char,15] | alternative primary therapy for gonorrhea | |
| TRMT2 | [Char,2] | treatment for presumptive chlamydial coinfection | 00=none, 01=ampicillin/amoxicillin, 09=doxycycline (Vibramycin)/tetracycline, 10=erythromycin, 11=azithromycin, 15=ofloxacin (Floxin), 88=other, 99=unknown |
| HISTORY | [Char,1] | previous history of gonorrhea | 1=yes, 2=no, 9=unknown |
| EPSDS | [Num,2] | number of previous episodes within the past 12 months | 0=no documented previous episodes in the past 12 months<br>99=unknown (patient record not available) |
| ZIP | [Char,5] | zipcode (residential) | 00000=homeless, 99999=unknown |

## GISP Data Elements

Antimicrobial Susceptibility Testing (Form 2 or CDC 73.60B)

| Variable Name | Type/Length | Description | Values |
|---|---|---|---|
| CLINIC | [Char,3] | Sentinel site code | ALB=Albuquerque, ANC=Anchorage, ATL=Atlanta, BAL=Baltimore, BHM=Birmingham, CHI=Chicago, CIN=Cincinnati, CLE=Cleveland, DEN=Denver, FBG=Fort Bragg, FLW=Fort Lewis, HON=Honolulu, KCY=Kansas City, LBC=Long Beach, MIA=Miami, MIN=Minneapolis, NAS=Nassau County, NOR=New Orleans, ORA=Orange County, PHI=Philadelphia, PHX=Phoenix, POR=Portland, SDG=San Diego, SEA=Seattle, SFO=San Francisco, STL=St. Louis, STO=San Antonio, WPB=West Palm Beach |
| ID | [Char,2] | patient or isolate number | 01, 02, 03, ... 25 |
| B_LAC | [Char,1] | beta-lactamase test | 1=positive, 2=negative |
| PEN | [Num,6] | penicillin MIC | 0.0008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0; 16.0; 32.0 |
| TETRACY | [Num,6] | tetracycline MIC | 0.06; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0; 16.0; 32.0 |
| SPCTINO | [Char,1] | spectinomycin sensitivity | 1=sensitive; 2=resistant |
| CFX | [Num,5] | cefixime MIC | 0.002; 0004; 0.008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0; 2.0 |
| CRO | [Num,5] | ceftriaxone MIC | 0.001; 0.002; 0.004; 0.008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0 |
| CIPRO | [Num,5] | ciprofloxacin MIC | 0.001; 0.002; 0.004; 0.008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0 |
| ERYT | [Num,5] | erythromycin MIC | 0.004; 0.008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0 |
| AZI | [Num,5] | azithromycin MIC | 0.008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0 |
| DATETEST | [Date,10] | date of isolate testing | MM/DD/YYYY |

## GISP Data Elements

Control Strain Susceptibility Testing (Form 3 or CDC 73.60C)

| Variable Name | Type/Length | Description | Values |
|---|---|---|---|
| LAB | [Char,3] | Regional laboratory | EMO=Atlanta regional lab<br>UAB=Birmingham regional lab<br>CLV=Cleveland regional lab<br>DHH=Denver regional lab<br>UWA=Seattle regional lab |
| STRAIN | [Char,9] | strain number | F-18<br>F-28<br>F-45<br>P681E<br>CDC 10328<br>CDC 10329 |
| B_LAC | [Char,1] | beta-lactamase test | 1=positive, 2=negative |
| PEN | [Num,6] | penicillin MIC | 0.0008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0; 16.0; 32.0 |
| TETRACY | [Num,6] | tetracycline MIC | 0.06; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0; 16.0; 32.0 |
| SPCTINO | [Char,1] | spectinomycin sensitivity | 1=sensitive; 2=resistant |
| CFX | [Num,5] | cefixime MIC | 0.002; 0004; 0.008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0; 2.0 |
| CRO | [Num,5] | ceftriaxone MIC | 0.001; 0.002; 0.004; 0.008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0 |
| CIPRO | [Num,5] | ciprofloxacin MIC | 0.001; 0.002; 0.004; 0.008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0 |
| ERYT | [Num,5] | erythromycin MIC | 0.004; 0.008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0 |
| AZI | [Num,5] | azithromycin MIC | 0.008; 0.015; 0.03; 0.06; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0 |
| DATETEST | [Date,10] | date of isolate testing | MM/DD/YYYY |

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